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RAMIREZ, DELIA M	
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Please find below and/or attached an Office communication concerning this application or proceeding.

The time period for reply, if any, is set in the attached communication.

Notice of the Office communication was sent electronically on above-indicated "Notification Date" to the following e-mail address(es):

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Office Action Summary	Application No.	Applicant(s)	
	10/539,560	YAMAKI ET AL.	
	Examiner	Art Unit	
	Delia M. Ramirez	1652	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE *3* MONTH(S) OR THIRTY (30) DAYS, WHICHEVER IS LONGER, FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *01 May 2008*.

2a)☐ This action is **FINAL**. 2b)☒ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *1-9,23-27,35-39 and 50-101* is/are pending in the application.

4a) Of the above claim(s) *23-27,35-39,50-54,56-71,73-76 and 85-101* is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *1-9,55,72 and 77-84* is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☒ The specification is objected to by the Examiner.

10)☒ The drawing(s) filed on *17 June 2005* is/are: a)☒ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

11)☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. § 119

12)☒ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☒ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☒ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☒ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☒ Information Disclosure Statement(s) (PTO/SB/08) Paper No(s)/Mail Date *6/17/05*.
4) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____ .
5) ☐ Notice of Informal Patent Application
6) ☒ Other: *alignments*.

DETAILED ACTION

Status of the Application

Claims 1-9, 23-27, 35-39 and 50-101 are pending.

Applicant's election with traverse of Group C, claims 2-6, 8-9, 55, 72, 77-84, in a communication filed on 1/28/2008, and election of positions 6, 19, and 126 of SEQ ID NO: 1 and positions 48, 108 and 212 of SEQ ID NO: 2 as submitted in a communication filed on 5/1/2008 are acknowledged.

Applicant traverses the rejection in a communication filed on 1/28/2008 on the grounds that (1) an alignment of SEQ ID NO: 1 with the sequences of the α subunits of the *R. rhodochrous* J1 nitrile hydratases of Kobayashi et al. shows either 43% or 52% sequence homology, thus even if substitutions would have been made at the alleged positions, there are many residues that are different, making the protein of Kobayashi et al. and that of the instant invention completely different, (2) the Examiner's comparison is not based on a comparison between before and after substitution but on comparison between the amino acids at the positions by the number corresponding to the substitution of the invention from the N-terminus of three kinds of native NHases, (3) Kobayashi et al. never disclosed that their protein was obtained from substituting amino acids in a known enzyme, (4) the level of homology between the proteins compared was ignored, and (5) proteins should have been aligned before comparison for proper analysis.

Applicant's arguments have been fully considered but are not deemed persuasive to withdraw the restriction requirement. To clarify the record, it should be noted that the 37th position indicated in the restriction requirement should have read 36th. The Examiner regrets this typographical error. However, it does not affect the analysis previously presented because both positions, i.e., 36th and 37th , in the α subunit of the J1-H nitrile hydratase of Kobayashi et al. contain valine residues. The Examiner acknowledges that (1) the nitrile hydratase H of

Kobayashi et al. and the wild-type nitrile hydratase of the instant application are from different organisms and that their α and β subunits are approximately 54% (111x100/205) and 34% (79x100/233) sequence identical, respectively, and (2) the proteins of Kobayashi et al. are isolated from nature and not the result of mutations made to a wild-type enzyme. However, it should be noted that the patentability of a product depends solely on its characteristics and not on how it is made. In the instant case, if (1) a wild-type protein X has amino acid sequence X and a man-made variant protein Y has an amino acid sequence which is identical to amino acid sequence X, and (2) the amino acid sequence of a protein determines its functional characteristics, it is unclear as to how one could differentiate between the wild-type protein X and the man-made protein Y if they are structurally and functionally identical.

With regard to the level of homology between the protein of Kobayashi et al. and that of the instant application, it should be noted that the claims do not limit how many modifications can be made to the subunit of SEQ ID NO: 1 or how much sequence identity should exist between the variant recited and the polypeptide of SEQ ID NO: 1. All that is required in claim 1 is that at least one of the specific recited positions in SEQ ID NO: 1 has to be different. The fact that claim 1 encompasses any number of modifications in addition to those recited is also evident in claim 2, as this claim further adds modifications to those recited in claim 1. With regard to arguments that proteins should have been aligned before comparison for proper analysis, it is noted that the claims do not require the identification and substitution of amino acids which would correspond to the amino acids at positions 36^{th}, 71^{th} or 148^{th} of the protein of SEQ ID NO: 1. The α subunit of the protein of Kobayashi et al. labeled as J1-H in Figure 4 is 203 amino acids and can be obtained by deleting the last two amino acids of the protein of SEQ ID NO: 1 and by directly substituting any of the positions in the protein of SEQ ID NO: 1 from amino acids 1-203 such that when all the substitutions are made, one would obtain a protein having the same amino acid sequence as that of Kobayashi et al. If one of skill in the art makes these direct

substitutions, the resulting protein would have many substitutions including (1) a valine residue at position 36th instead of the threonine residue at position 36th of SEQ ID NO: 1, a threonine residue at position 71th instead of an arginine residue at position 71th of SEQ ID NO: 1, and a glutamic acid residue at position 148th instead of a glycine residue at position 148th of SEQ ID NO: 1. Therefore, the α subunit of the protein of Kobayashi et al. labeled as J1-H meets the structural and functional requirements of claim 1. Furthermore, even if one were to align the α subunit of the protein of Kobayashi et al. and the polypeptide of SEQ ID NO: 1 taking into consideration those motifs which are associated with nitrile hydratase activity, the polypeptide of Kobayashi et al. would have a proline residue at a position corresponding to position 36th of SEQ ID NO: 1, and a tryptophan residue at a position corresponding to position 71th of SEQ ID NO: 1. See attached alignment. Thus, even if the proteins are aligned as Applicant suggests, the α subunit of the protein of Kobayashi et al. labeled as J1-H meets the limitations of claim 1. As such, the technical feature linking the claimed inventions does not make a contribution over the prior art and the claimed invention does not meet the requirement of unity of invention under PCT Rule 13.2

The requirement is deemed proper and therefore is made FINAL.

At the present time, linking claims 1 and 7 are not allowable. Thus the previous restriction requirement can be properly maintained. Claims 23-27, 35-39, 50-54, 56-71, 73-76 and 85-101 are withdrawn from further consideration by the Examiner, 37 CFR 1.142(b), as being drawn to a non-elected invention. Claims 2-6, 8-9, 55, 72, 77-84 will be examined to the extent they are directed to the elected invention. Claims 1-9, 55, 72 and 77-84 are at issue and are being examined herein.

Specification

1. The abstract of the disclosure is objected to because it does not contain the proper language/format. See MPEP § 608.01(b). Applicant is reminded of the proper language and format for an abstract of the disclosure. The abstract should be in narrative form and generally limited to a single paragraph on a separate sheet within the range of 50 to 150 words. It is important that the abstract not exceed 150 words in length since the space provided for the abstract on the computer tape used by the printer is limited. The form and legal phraseology often used in patent claims, such as "means" and "said," should be avoided. The abstract should describe the disclosure sufficiently to assist readers in deciding whether there is a need for consulting the full patent text for details. Correction is required.

2. The title of the invention is not descriptive. A new title is required that is clearly indicative of the invention to which the claims are directed. It is suggested the title be amended to include the source of the nitrile hydratase or some identifying characteristic which differentiates it from other nitrile hydratases.

Priority

3. Acknowledgment is made of a claim for foreign priority under 35 U.S.C. 119(a)-(d) to JAPAN 2003-379280 filed on 11/10/2003, and JAPAN 2002368360 filed on 12/19/2002.

Receipt is acknowledged of papers submitted under 35 U.S.C. 119(a)-(d), which papers have been placed of record in the file. No English translation of these priority documents has been filed.

4. This application is the US national stage of PCT/JP03/16014 filed on 12/15/2003.

Information Disclosure Statement

5. The information disclosure statement (IDS) submitted on 6/17/2005 is acknowledged. The submission is in compliance with the provisions of 37 CFR 1.97. Accordingly, the information disclosure statement is being considered by the examiner.

Drawings

6. The drawings submitted on 6/17/2005 have been reviewed and are accepted by the Examiner for examination purposes.

Claim Objections

7. Claims 2-5, 7-9, 55, 72, 77-84 are directed in part to non-elected inventions. Examination of such claims will be restricted to the subject matter elected. Applicant is requested to amend the claims accordingly in response to this Office Action.

8. Claims 1, 3, 7 and 9 are objected to due to the recitation of "in the Sequence Listing". This is deemed redundant since there is no other SEQ ID NO: 1 or 2 in the disclosure but those of the Sequence Listing. Appropriate correction is required.

9. Claim 55 is objected to under 37 CFR 1.75(c) as being in improper form because a multiple dependent claim cannot depend upon another multiple dependent claim. See MPEP § 608.01(n). In the instant case, claim 4 is deemed a multiple dependent claim by virtue of its dependency on claim 3. Claim 55 depends on claim 4. For examination purposes, it will be

assumed that claim 55 reads "any one of claims 1, 2, 6, 7 or 8". Appropriate correction is required.

Claim Rejections - 35 USC § 101

10. 35 U.S.C. 101 reads as follows:

> Whoever invents or discovers any new and useful process, machine, manufacture, or composition of matter, or any new and useful improvement thereof, may obtain a patent therefor, subject to the conditions and requirements of this title.

11. Claims 1-9, 82-84 are rejected under 35 U.S.C. 101 because the claimed invention is directed to non-statutory subject matter.

Claims 1-9, 82-84, as written, do not sufficiently distinguish over proteins as they exist naturally because the claim(s) does not particularly point out any non-naturally occurring differences between the claimed product and the naturally occurring product. It should be noted that there are naturally-occurring mutations. In the absence of the hand of man, the naturally occurring products are considered non-statutory subject matter. See *Diamond v. Chakrabarty*, 447 U.S. 303, 206 USPQ 193 (1980). The claim(s) should be amended to indicate the hand of the inventor, e.g., by insertion of "isolated" or "purified" as taught by Example 74 of the specification. See MPEP 2105.

Claim Rejections - 35 USC § 112, Second Paragraph

12. The following is a quotation of the second paragraph of 35 U.S.C. 112:

> The specification shall conclude with one or more claims particularly pointing out and distinctly claiming the subject matter which the applicant regards as his invention.

13. Claims 1-9, 55, 72, 77-84 are rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

14. Claims 1 and 7 (claims 2-6, 8-9, 55, 82-84 dependent thereon) are indefinite in the recitation of "a nitrile hydratase comprising an α subunit and a β subunit wherein the α/ β subunit has an amino acid sequence in which at least one amino acid of the X^{th} amino acids in the amino acid sequence of SEQ ID NO: 1/2 in the Sequence Listing is substituted by another amino acid" for the following reasons. The amino acid sequence of the subunit is not that of SEQ ID NO: 1 or 2. Instead, the amino acid sequence of the subunit is a variant of SEQ ID NO: 1 or 2. While the term "wherein the ..subunit has an amino acid sequence in which at least one amino acid" indicates that the "at least one amino acid" refers to an amino acid from the amino acid sequence of the subunit, the term " X^{th} amino acids in the amino acid sequence of SEQ ID NO: 1/2" refers to amino acids which are in SEQ ID NO: 1 or 2. This is unclear and confusing. For examination purposes, it will be assumed that the claims read "a nitrile hydratase comprising an α subunit and a β subunit, wherein the α/ β subunit is a variant of the polypeptide of SEQ ID NO: 1/2, wherein said variant is obtained by substituting at least one of the amino acids of positions X, Y, or Z of SEQ ID NO: 1/2 for another amino acid". Correction is required.

15. Claims 2, 4, 5, 8, 82, 83 are indefinite in the recitation of "wherein at least one amino acid of the X^{th} amino acids in the amino acid sequence of the …subunit is further substituted" for the following reasons. Claims 1 and 7, from which these claims depend, refer to specific positions within SEQ ID NO: 1 or 2. The instant claims as written appear to refer to positions of the subunits which are variants of the polypeptides of SEQ ID NO: 1 or 2. Thus, it is unclear if the intended positions are those of SEQ ID NO: 1 or 2, as recited in the claims from which they depend. For examination purposes, it will be assumed that the claims read "the nitrile hydratase of claim X wherein at least one of the amino acids at positions A, B, or C of SEQ ID NO: 1/2 is further substituted by another amino acid. Correction is required.

16. Claims 4 and 82 (claims 5 and 83 dependent thereon) are indefinite in the recitation of "the nitrile hydratase according to claim 3/9 wherein at least one amino acid of the ...of the α/β

subunit is substituted by another amino acid" for the following reasons. Claims 3 and 9, from which claims 4 and 82 define the α/β subunits as those having SEQ ID NO: 1/2. As such, the amino acid sequences of these subunits have been defined. Claims 4 and 82 broaden the scope of claims 3 and 9 by including variants of the α/β subunits recited. For examination purposes, it will be assumed that claims 4 and 82 are independent claims which include the limitations of claims 3 and 9, respectively. Correction is required.

17. Claims 6 and 84 are indefinite in the recitation of "wherein at sites other than the amino acid substitution sites in the amino acid sequence carried by at least one of the β and the α subunit, one or several amino acids are subject to substitution, insertion or deletion within the scope of not impairing the nitrile hydratase activity" for the following reasons. Claim 1, from which claim 6 depends, only recites substitutions to the α subunit, therefore the term "amino acid substitution sites in the amino acid sequence carried by….the β subunit " is unclear and confusing. Similarly, claim 7, from which claim 84 depends, only recites substitutions to the β subunit, therefore the term "amino acid substitution sites in the amino acid sequence carried by….the α subunit " is unclear and confusing. Furthermore, it is unclear as to what is the intended meaning of the term "within the scope of not impairing the nitrile hydratase activity". It should be noted that claims 1 and 7 both require that the protein claimed be a nitrile hydratase. Also, the proteins of claims 1 and 7 are not limited with regard to the modifications that can be made to those proteins in addition to those recited. Thus, in addition to the deficiencies described above, it is unclear as to how these claims further limit claims 1 and 7. For examination purposes, claims 6 and 84 will be considered duplicates of claims 1 and 7, respectively. Correction is required.

18. Claims 55, 72, 77-81 are indefinite in their reference to non-elected method claims or their reference to non-elected product claims for the following reasons. The method/product claims recited are dependent upon other method claims. Thus, as written, it is unclear as to which

limitations should be read into the claims. For examination purposes, no patentable weight will be given to the recitation of other non-elected claims. Claims 55 and 77 will be interpreted as methods of use of any nitrile hydratase, whereas claims 72, 78-81 will be interpreted as directed to any nitrile hydratase. Correction is required.

Claim Rejections - 35 USC § 112, First Paragraph

19. The following is a quotation of the first paragraph of 35 U.S.C. 112:

> The specification shall contain a written description of the invention, and of the manner and process of making and using it, in such full, clear, concise, and exact terms as to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the same and shall set forth the best mode contemplated by the inventor of carrying out his invention.

20. Claims 1-9, 55, 72, 77-84 are rejected under 35 U.S.C. 112, first paragraph, as failing to comply with the written description requirement. The claim(s) contains subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventor(s), at the time the application was filed, had possession of the claimed invention.

Claims 1-9, 55, 72, 77-84 require nitrile hydratases comprising essentially any structure. It should be noted that even those claims reciting substitutions at different positions within SEQ ID NO: 1 and/or 2 require proteins having virtually any structure in view of the fact that those claims are not limited with regard to the number of substitutions that can be made, nor do they define the actual residue which should be in place of the substituted amino acid. As such, the claims require proteins which have any structure. See also Claim Rejections under 35 USC 112, second paragraph for claim interpretation.

In *University of California v. Eli Lilly & Co.*, 43 USPQ2d 1938, the Court of Appeals for the Federal Circuit has held that "A written description of an invention involving a chemical genus, like a description of a chemical species, 'requires a precise definition, such as by

structure, formula, [or] chemical name,' of the claimed subject matter sufficient to distinguish it from other materials". As indicated in MPEP § 2163, the written description requirement for a claimed genus may be satisfied through sufficient description of a representative number of species by actual reduction to practice, reduction to drawings, or by disclosure of relevant, identifying characteristics, i.e., structure or other physical and/or chemical properties, by functional characteristics coupled with a known or disclosed correlation between function and structure, or by a combination of such identifying characteristics, sufficient to show that Applicant was in possession of the claimed genus. In addition, MPEP § 2163 states that a representative number of species means that the species which are adequately described are representative of the entire genus. Thus, when there is substantial variation within the genus, one must describe a sufficient variety of species to reflect the variation within the genus.

There is no actual structural limitation with regard to the members of the genus of proteins recited. While the specification in the instant application discloses the structure of a single wild type nitrile hydratase having the α subunit of SEQ ID NO: 1 and the β subunit of SEQ ID NO: 2, as well as several mutants of the α subunit of SEQ ID NO: 1 and the β subunit of SEQ ID NO: 2, the specification provides no clue as to the structural elements required in any protein having nitrile hydratase activity, nor does it teach which structural elements of the polypeptide of SEQ ID NO: 1 and 2 are required in any protein having nitrile hydratase activity.

The claim encompasses a large genus of proteins which is structurally unrelated. A sufficient written description of a genus of polypeptides may be achieved by a recitation of a representative number of polypeptides defined by their amino acid sequence or a recitation of structural features common to members of the genus, which features constitute a substantial portion of the genus. However, in the instant case, there is no structural feature which is representative of all the members of the genus of proteins recited in the claim, and there is no information as to a correlation between structure and function. Furthermore, while one could

argue that SEQ ID NO: 1 and 2 are representative of the structure of all the members of the genus of α and β subunits of a nitrile hydratase, such that the recited genus of nitrile hydratases is adequately described, it is noted that the art teaches several examples of how even small structural variation can lead to changes in function. For example, Witkowski et al. (Biochemistry 38:11643-11650, 1999) teach that one conservative amino acid substitution transforms a β-ketoacyl synthase into a malonyl decarboxylase and completely eliminates β-ketoacyl synthase activity. Seffernick et al. (J. Bacteriol. 183(8):2405-2410, 2001) teach that two naturally occurring *Pseudomonas* enzymes having 98% amino acid sequence identity catalyze two different reactions: deamination and dehalogenation, therefore having different function. Therefore, since minor structural changes to a polypeptide may result in changes affecting function, and no additional information correlating structure with nitrile hydratase activity has been provided, one cannot reasonably conclude that SEQ ID NO: 1 and 2, or variants thereof as taught by the specification, are representative of the structure of all nitrile hydratases as recited.

Due to the fact that the specification only discloses one wild-type nitrile hydratase and a few mutants of the α and β subunits, as well as the lack of description of any additional species by any relevant, identifying characteristics or properties, one of skill in the art would not recognize from the disclosure that Applicant was in possession of the claimed invention.

21. Claims 1-9, 55, 72, 77-84 are rejected under 35 U.S.C. 112, first paragraph, because the specification, while being enabling for (1) a nitrile hydratase comprising an α and a β subunit, wherein the α subunit is a variant of the polypeptide of SEQ ID NO: 1 which differs from the polypeptide of SEQ ID NO: 1 solely by amino acid substitutions at positions 6, 36, 71, 148, 204, 19, and/or 126, and wherein the β subunit is a variant of the polypeptide of SEQ ID NO: 2 which differs from the polypeptide of SEQ ID NO: 2 solely by amino acid substitutions at positions 48, 108, and/or 212, and (2) a method for producing an amide compound by contacting a nitrile

compound with the nitrile hydratase of (1), does not reasonably provide enablement for a nitrile hydratase having any structure, or a method of use of said nitrile hydratase for the production of amide compounds. The specification does not enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and/or use the invention commensurate in scope with these claims.

Factors to be considered in determining whether undue experimentation is required are summarized in *In re Wands* (858 F.2d 731, 737, 8 USPQ2nd 1400 (Fed. Cir. 1988)) as follows: 1) quantity of experimentation necessary, 2) the amount of direction or guidance presented, 3) the presence and absence of working examples, 4) the nature of the invention, 5) the state of prior art, 6) the relative skill of those in the art, 7) the predictability or unpredictability of the art, and 8) the breath of the claims. The factors which have lead the Examiner to conclude that the specification fails to teach how to make and/or use the claimed invention without undue experimentation, are addressed in detail below.

The breath of the claims. Claims 1-9, 55, 72, 77-84 are so broad as to encompass essentially any protein having nitrile hydratase activity, and a method to produce amides by contacting said protein with a nitrile. The enablement provided is not commensurate in scope with the claim due to the extremely large number of proteins of unknown structure required by the claims. In the instant case, the specification enables (1) a nitrile hydratase comprising an α and a β subunit, wherein the α subunit is a variant of the polypeptide of SEQ ID NO: 1 which differs from the polypeptide of SEQ ID NO: 1 solely by amino acid substitutions at positions 6, 36, 71, 148, 204, 19, and/or 126, and wherein the β subunit is a variant of the polypeptide of SEQ ID NO: 2 which differs from the polypeptide of SEQ ID NO: 2 solely by amino acid substitutions at positions 48, 108, and/or 212, and (2) a method for producing an amide compound by contacting a nitrile compound with the nitrile hydratase of (1).

The amount of direction or guidance presented and the existence of working examples. The specification discloses the amino acid sequences of the α and β subunits of a single nitrile hydratase as a working example (SEQ ID NO: 1 and 2). The specification also discloses a few mutations made to the α and β subunits of SEQ ID NO: 1 and 2, respectively. However, the specification fails to provide any clue as to the structural elements required in any protein having nitrile hydratase activity, or which are the structural elements within the polypeptides of SEQ ID NO: 1 and 2 which are essential for any protein to display nitrile hydratase activity. No correlation between structure and function has been presented.

The state of prior art, the relative skill of those in the art, and the predictability or unpredictability of the art. The amino acid sequence of a polypeptide determines its structural and functional properties. While the art discloses a few proteins having nitrile hydratase activity, neither the specification nor the art provide a correlation between structure and nitrile hydratase activity such that one of skill in the art can envision the structure of any protein having nitrile hydratase activity. In addition, the art does not provide any teaching or guidance as to the general tolerance of nitrile hydratases to structural modifications and the extent of such tolerance. The art clearly teaches that modification of a protein's amino acid sequence to obtain the desired activity without any guidance/knowledge as to which amino acids in a protein are tolerant of modification and which ones are conserved is highly unpredictable. At the time of the invention there was a high level of unpredictability associated with altering a polypeptide sequence with an expectation that the polypeptide will maintain the desired activity. For example, Branden et al. (Introduction to Protein Structure, Garland Publishing Inc., New York, page 247) teach that (1) protein engineers are frequently surprised by the range of effects caused by single mutations that they hoped would change only one specific and simple property in enzymes, (2) the often surprising results obtained by experiments where single mutations are made reveal how little is known about the rules of protein stability, and (3) the difficulties in designing *de novo* stable

proteins with specific functions. The teachings of Branden et al. are further supported by the teachings of Witkowski et al. (Biochemistry 38:11643-11650, 1999) and Seffernick et al. (J. Bacteriol. 183(8):2405-2410, 2001) already discussed above, where it is shown that even small amino acid changes result in enzymatic activity changes.

The quantity of experimentation required to practice the claimed invention based on the teachings of the specification. While methods of generating or isolating variants of a polypeptide were known in the art at the time of the invention, it was not routine in the art to screen by a trial and error process for all polypeptides having nitrile hydratase activity. In the absence of (1) a rational and predictable scheme for modifying any residue in the polypeptides of SEQ ID NO: 1 and 2 such that the resulting variants when in contact with each other would display nitrile hydratase activity, and/or (2) a correlation between structure and nitrile hydratase activity, one of skill in the art would have to test an essentially infinite number of proteins to determine which ones have nitrile hydratase activity.

Therefore, taking into consideration the extremely broad scope of the claim, the lack of guidance, the amount of information provided, the lack of knowledge about a correlation between structure and the desired function, and the high degree of unpredictability of the prior art in regard to structural changes and their effect on function, one of ordinary skill in the art would have to go through the burden of undue experimentation in order to practice the claimed invention. Thus, Applicant has not provided sufficient guidance to enable one of ordinary skill in the art to make and use the invention in a manner reasonably correlated with the scope of the claims.

Claim Rejections - 35 USC § 102

22. The following is a quotation of the appropriate paragraphs of 35 U.S.C. 102 that form the basis for the rejections under this section made in this Office action:

> A person shall be entitled to a patent unless -
>
> (b) the invention was patented or described in a printed publication in this or a foreign country or in public use or on sale in this country, more than one year prior to the date of application for patent in the United States.

23. Claims 1-2, 4-8, 55, 72, 77-82-84 are rejected under 35 U.S.C. 102(b) as being anticipated by Kobayashi et al. (Biochimica et Biophysica Acta 1129:23-33, 1991; cited in the IDS).

Claims 1-2, 4-8, 82-84 are directed in part to any nitrile hydratase wherein said nitrile hydratase comprises an α and a β subunit, wherein the α subunit is a variant of the polypeptide of SEQ ID NO: 1 having amino acid substitutions at positions 36, 71, 148, 6, 19, and 126 of SEQ ID NO: 1, and wherein the β subunit is a variant of the polypeptide of SEQ ID NO: 2 having amino acid substitutions at positions 48, 108 and 212 of SEQ ID NO: 2. Claims 72, 78-81 are directed to any nitrile hydratase wherein said nitrile hydratase comprises an α and a β subunit. Claims 55 and 77 are directed in part to a method for the production of an amide wherein said method comprises contacting a nitrile with any nitrile hydratase in an aqueous medium or in the presence of a solvent. See Claim Rejections under 35 USC 112, second paragraph, for claim interpretation.

Kobayashi et al. teach a *R. rhodochrous* J1 nitrile hydratase H having an α and a β subunit (Abstract; Figure 4, top line) as well as a method for producing benzamide (amide) from benzonitrile (nitrile) in the presence of a phosphate buffer (aqueous medium, water is the solvent) and the nitrile hydratase (page 25, right column, Enzyme assays). The α subunit of the nitrile hydratase of Kobayashi et al. (203 amino acids) is a variant of the polypeptide of SEQ ID NO: 1 (205 amino acids) wherein said variant is the result of deleting the last two amino acids of the polypeptide of SEQ ID NO: 1, and substituting several amino acids within SEQ ID NO: 1 from amino acids 1-203, including substitutions at positions 36, 71, 148, 6, 19 and 126 of SEQ ID NO: 1 as follows: the threonine at position 36 has been substituted with valine, the arginine at position 71 has been substituted with threonine, the glycine at position 148 has been substituted with

glutamic acid, the leucine at position 6 has been substituted with asparagine, the alanine at position 19 has been substituted with glutamic acid, and the phenylalanine at position 126 has been substituted with arginine. See Figure 4, α subunit, first line. Also, if the α subunit of the nitrile hydratase of Kobayashi et al. is aligned with the polypeptide of SEQ ID NO: 1 to take into consideration conserved motifs, the α subunit of the nitrile hydratase of Kobayashi et al. is a variant of the polypeptide of SEQ ID NO: 1 wherein the position corresponding to position 36 of SEQ ID NO: 1 has been substituted with a proline, the position corresponding to position 71 of SEQ ID NO: 1 has been substituted with tryptophan, the position corresponding to position 126 of SEQ ID NO: 1 has been substituted with a tyrosine. See attached alignment.

The β subunit of the nitrile hydratase of Kobayashi et al. (229 amino acids) is a variant of the polypeptide of SEQ ID NO: 2 (233 amino acids) wherein said variant is the result of deleting the last four amino acids of the polypeptide of SEQ ID NO: 2, and substituting several amino acids within SEQ ID NO: 2 from amino acids 1-229, including substitutions at positions 10, 32, 37, 41, 46, 48, 51, 72, 108, 118, 146, 186, 212 and 217 of SEQ ID NO: 2 as follows: the threonine at position 10 has been substituted with methionine, the valine at position 32 has been substituted with arginine, the phenylalanine at position 37 has been substituted with leucine, the phenylalanine at position 41 has been substituted with histidine, the methionine at position 46 has been substituted with serine, the leucine at position 48 has been substituted with tryptophan, the phenylalanine at position 51 has been substituted with serine, the tryptophan at position 72 has been substituted with tyrosine, the glutamic acid at position 108 has been substituted with aspartic acid, the phenylalanine at position 118 has been substituted with alanine, the arginine at position 146 has been substituted with aspartic acid, the leucine at position 186 has been substituted with serine, the serine at position 212 has been substituted with asparagine, and the aspartic acid at position 217 has been substituted with valine. See Figure 4, β subunit, first line. Also, if the β subunit of the nitrile hydratase of Kobayashi et al. is aligned with the polypeptide of SEQ ID

NO: 2 to take into consideration conserved motifs, the β subunit of the nitrile hydratase of Kobayashi et al. is a variant of the polypeptide of SEQ ID NO: 2 wherein the position corresponding to position 10 of SEQ ID NO: 2 has been substituted with methionine, the position corresponding to position 32 of SEQ ID NO: 2 has been substituted with arginine, the position corresponding to position 37 of SEQ ID NO: 2 has been substituted with leucine, the position corresponding to position 41 of SEQ ID NO: 2 has been substituted with histidine, the position corresponding to position 46 of SEQ ID NO: 2 has been substituted with serine, the position corresponding to position 48 of SEQ ID NO: 2 has been substituted with tryptophan, the position corresponding to position 51 of SEQ ID NO: 2 has been substituted with serine, the position corresponding to position 108 of SEQ ID NO: 2 has been substituted with aspartic acid, the position corresponding to position 118 of SEQ ID NO: 2 has been substituted with arginine, the position corresponding to position 146 of SEQ ID NO: 2 has been substituted with lysine, the position corresponding to position 160 of SEQ ID NO: 2 has been substituted with lysine, and the position corresponding to position 212 of SEQ ID NO: 2 has been substituted with aspartic acid. See attached alignment.

Therefore, the nitrile hydratase and method of Kobayashi et al. anticipate the instant claims as written/interpreted.

Conclusion

24. No claim is in condition for allowance.

25. Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PMR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see http://pair-direct.uspto.gov. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free).

26. Any inquiry concerning this communication or earlier communications from the examiner should be directed to Delia M. Ramirez whose telephone number is (571) 272-0938. The examiner can normally be reached on Monday-Friday from 9:30:00 AM to 6:00 PM. If

attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Dr. Nashaat Nashed can be reached on (571) 272-0934. Any inquiry of a general nature or relating to the status of this application or proceeding should be directed to the receptionist whose telephone number is (571) 272-1600.

/Delia M. Ramirez/

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